

Role of Isoflavones on Lipid Peroxidation, Superoxide Dismutase in Lymphocytes under Oxidative Stress Conditions

I Nyoman Suarsana^{1*}, I Nyoman Mantik Astawa², I Made Kardena³

¹Laboratory Biochemistry, ²Laboratory Virology and immunology, ³Laboratory Pathology

Faculty of Veterinary Medicine, University of Udayana, Bali, Indonesia

*E-mail of the corresponding author: suarsana65@yahoo.com

Abstract

Oxidative stress conditions forms free radicals in cells. Free radicals react continuously and cause cell membranes damage including lymphocytes, thus decreasing cell function. Therefore, there were efforts need to be done to overcome abnormalities and decreased lymphocyte cell function under conditions of oxidative stress. One of them using bioactive compounds that have antioxidant activity, such as isoflavones. Isoflavones have been reported to have antioxidant activity and the potential to protect cells of oxidative stress. This study aims to determine the role of isoflavones as antioxidative in protecting lymphocytes under conditions of oxidative stress, be reviewed of CD4, CD8, malondialdehyde levels and superoxide dismutase activity. This study was used rat lymphocyte cell cultures in two conditions, i.e: oxidative stress and without oxidative stress. The cell culture were treated with 20 ml of 2 mM paraquat and isoflavone at dose 0, 1000, 2000, and 3000 ppm, respectively. The parameters were observed: (1) malondialdehyde (MDA) levels, (2) superoxide dismutase (SOD) activity, and (3) percentage of CD4 and CD8 lymphocytes cell using a monoclonal anti-rat CD4 and CD8 FITC-conjugate. The results showed paraquat administration in cell culture on oxidative stress conditions could reduce the number of CD4 and CD8 lymphocytes cells, increased of malondialdehyde (MDA) and superoxide dismutase (SOD) activity. Administration of isoflavones on culture that treated without oxidative stress condition could increase percentage number of CD4, CD8, MDA levels, and SOD activity. Whereas, administration of isoflavones on oxidative stress condition prevented the decrease percentage number of CD4, CD8, MDA levels, and SOD activity. Administration of isoflavone at dose 3000 ppm in cell culture that treated on oxidative stress condition provided optimal results in preventing the decrease number of CD4, CD8 lymphocytes cell, MDA levels, and SOD activity.

Keywords: isoflavone, lymphocytes, CD4, CD8, malondialdehyde, superoxide dismutase.

1. Introduction

Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) production and the antioxidant defense mechanisms of a biological organism that results directly or indirectly in cellular damage (Jenkins, 2000; Fisher, 2010; Fisher *et al.*, 2011). Sources of ROS can be from exogenous or endogenous origins of the body. ROS from exogenous origins (chemicals, radiation, air pollutants, intoxication by oxygen, smoke, and alcohol) and from endogenous origins such as oxidative phosphorylation within the mitochondria or extramitochondrial sources (Jezek and Hlavata, 2005; Fisher, 2010). ROS are recognized for harmful to living systems. At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko *et al.*, 2007).

Exposure of the body from the toxic or harmful substances are carcinogenic, immunotoxic, and other toxicant compound which can harmful to health. According Krejsa and Schieven (2000), toxicant compounds can induce oxidative stress by various mechanisms. This toxicant compounds can block the electron transport chain thereby increasing leakage of free radicals such as superoxide anion ($O_2^{\bullet-}$), from the mitochondria to the cytosol. Paraquat (PQ) is one of the toxicant compound. It is a herbicide that works fast, it is not selective and it is toxic to all cells (Ogamba *et al.*, 2011). According to Raina *et al.* (2008), paraquat can inhibit the reduction of NADP to NADPH. This disorder causes formation of Free radicals.

Free radicals can injury all cells inclusive lymphocytes by destructing which injury cellular lipids, proteins, or DNA (Zakaria *et al.*, 1997a). Therefore, normal function of the cells is inhibited. Oxidative stress condition can cause degenerative diseases, immune system disorders and cells premature aging.

The main components of the specific immune system are lymphocyte. Lymphocytes are an important immunological cell and have been played a significant role in acquired immune system (Gautam *et al.*, 2010). The immune system consists of a non-specific immune system (physical, mechanical and chemical) and specific immunity (cellular) (Kelly, 2007).

According to Tood (2001), there are three major types of lymphocytes are called B cells, T cells and NK (natural killer) cells. T cells expressing the surface proteins CD8 (helper T cells) or CD4 (cytotoxic cells), respectively. T cells consists of three subsets, namely helper T cells (T_H), suppressor T cells (T_S), and cytotoxic T cells (T_C). Each of these subsets of T cells expressing surface markers or cluster of differentiation (CD)..

Free radicals can damage cell membrane of lymphocytes which are characterized by the increasing lipid peroxidation products, decrease intracellular antioxidant activity (SOD) and lymphocyte loses its primary function, which is not able to eliminate a variety of agents that entry into the body. Various studies have shown that oxidative stress can impact on immune function (Shi *et al.*, 2003). The immune system is affected by stress, for instance: physical stress, chemical stress, and oxidative stress. Stress that occurs chronically, may cause death (apoptosis) cell lymphocytes.

Isoflavones are polyphenol compounds that commonly contain in soybeans and it's products such as tempe. Tempe is a widely consumed by Indonesians. It's traditional fermented food, which is principally made with soybeans and rich of isoflavone compound (Astuti *et al.*, 2000). Isoflavones has been reported to have antioxidant activities (Lee *et al.*, 2004). In addition, the polyphenols contained in food and plants have been reported to stimulate the immune response (immunomodulator) (Zakaria *et al.*, 1997). Isoflavones is an important factor an antioxidants and immunomodulator maintaining survival and function of lymphocytes in body's defense. This study aims were to determine role of isoflavones as antioxidative of the CD4, CD8, lipid peroxidation, superoxide dismutase in lymphocytes under conditions of oxidative stress.

2. Materials and Methods

Preparation of lymphocytes. Isolation of lymphocyte cells using method that modified by Mantik *et al* (2005). A total of 5 ml blood of rats inserted into a tube that had been contained anticoagulant (EDTA). Then the tube was centrifuged at a speed of 2500 rpm for 10 minutes. A white layer (buffy coat) in between the red blood cells and plasma was taken and suspended in medium without serum. Lymphocytes were separated from buffy coat with density gradient centrifugation, Ficoll-paque speed of 3000 rpm for 45 minutes. Lymphocyte layer was taken and washed three times with a medium without serum. Lymphocyte cells then were washed up to lymphocytes free of red blood cells. Finally, lymphocytes diluted with 5 ml RPMI 1640 and counted using a hemocytometer.

Lymphocyte cell culture. Rat is lymphocyte cells that had been counted then arranged into a cell containing 1×10^5 cells/ml using RPMI medium which was equipped with a 10% Fetal Calf Serum (FCS), penicillin (100 IU/ml), streptomycin (100 $\mu\text{g/ml}$), gentamicin (50 $\mu\text{g/ml}$) and 2.5 $\mu\text{g/ml}$ fungison. Every 100 ml of lymphocyte cells (1×10^5 cells / ml) in complete medium inserted randomly into the microplate wells then successively added to each well of a 20 μl isoflavone compounds that had been prepared with a concentration of 1000, 2000 and 3000 $\mu\text{g/ml}$ and added 20 μl of 2mM paraquat. For a positive control in oxidative stress, other microplate wells were added lymphocyte cells containing 1×10^5 cells/ml on complete medium, and than added of 20 μl in 2mM paraquat. For a positive control of isoflavone, each microplate wells was added 100 μl lymphocyte cells 1×10^5 cells/ml in complete medium and then added 20 μl isoflavone concentrations of 1000, 2000 and 3000 $\mu\text{g/ml}$ in every wells. All cultures in microplate wells were incubated for 24 hours at 37°C with 5% of CO_2 . Isoflavones were extracted from tempe and it's contain levels had been analyzed adapted form Suarsana *et al* (2010)

Analysis of lymphocyte cells. Analysis of CD4 and CD8 lymphocyte cells were evaluated by using direct staining method based on the procedures in the KIT. Monoclonal antibodies conjugated with the types of dyes fluorescens was used for staining. A total of 100 μl of lymphocyte cell suspension containing of 1×10^6 cells/ml was placed into a tube (falcon tube). The tube was added 4 μl of monoclonal antibody anti-mouse CD4-FITC conjugated (or CD8-FITC) and vortexed slowly. After it mixed, the suspension incubation for 35 min at 4°C. A total of 20 μl of cell suspension dropped on the object glass (microscope slide) and

and viewed with a microscope. Total cells that emitted yellowish-green fluorescens were counted under a microscope fluorescens and the lymphocytes were counted by using phase contrast microscopy. The results were expressed in percent of total cells, namely the number of fluorescent cells to total lymphocyte cells

Analysis of superoxide dismutase (SOD) activity. Analysis of SOD activity was conducted by using a method adapted from Nebot *et al.* (1993). A total of 100 μl lymphocyte supernatant and 400 μl solution of chloroform/cold ethanol (v/v: 37.5/62.5) added into Eppendorf tubes, and then homogenized for 3 seconds and centrifuged at 4000 rpm 4°C for 10 minutes. A 50 μl lysate solution (distilled water for control) was added 2.9 ml of a xanthine and cytochrome c (ratio 1:10) mixture and homogenized. Subsequently, the mixture was added

50 µl solution of xanthine oxidase and homogenized. Absorbance values was read using a spectrophotometer at λ 550 nm.

Analysis of lipid peroxidation (malondialdehyde). Lipid peroxidation (malondialdehyde) levels were analyzed using method addapted from Capeyron *et al.* (2002). A total of 0.5 ml of the supernatant or standard plus with 2 ml of 0.25 N HCl (cold) mixture containing 15%TCA, TBA 0.38%, and 0.5% BHT. The solution mixture was heated to 80°C for 1 hour. After the cold, solution was centrifuged 3500 rpm for 10 minutes. Absorbance values was read at λ 532 nm. As standard solution used TEP (1,1,3,3-tetraetoksipropana).

3. Results

The results analysis of CD4, CD8 T-cells, malondialdehyde levels and superoxide dismutase activity of lymphocyte cells under conditions of oxidative stress are shown in the Table 1-4.

The results of the percentage of CD4 T-cells in a different culture conditions and was given isoflavones are presented in Table 1. Analysis of variance showed that treatment of culture conditions (stress and without stress) and isoflavone treatment significantly effect ($P < 0.05$) to amount of CD4 lymphocyte cells.

Table 1. The number of CD4 lymphocyte cells that cultured under oxidative stress and without oxidative stress condition.

Culture condition	CD4 percentage (%) at different doses of isoflavones (ppm)				
	0	1000	2000	3000	Average
Non-stress	19.64Aa	21.56Aab	23.91Ab	25.65Ac	22.69A
Stress	11.42Ba	13.15Bab	15.58Bb	18.38Bc	14.63B
Average	15.53	17.36	19.74	22.01	

Description: Values with the same letter towards column (uppercase) and the values with the same letter in the direction of the line (lowercase) indicates not significantly different ($P > 0.05$)

The analysis indicated that the amount of CD8 T-cells condition oxidative stress was lower than the condition without oxidative stress and significantly different ($P < 0.05$) at all doses of isoflavones (Table 2). This suggests that paraquat administration significantly reduced the amount of CD8 T cells. On the condition of oxidative stress, the use of isoflavones at dose up to 3000 ppm resulted the amount of CD8 T cells was 19.65 while the amount of CD4 T cells without stress was 21.15.

Table 2. The percentage of CD8 T-cells that cultured under oxidative stress and without oxidative stress condition.

Culture condition	CD8 percentage (%) at different doses of isoflavones (ppm)				
	0	1000	2000	3000	Average
Non-stress	21.15Aa	22.45Aa	25.76Ab	27.42Ab	24.19A
Stress	11.93Ba	13.79Ba	16.97Bb	19.65Bb	15.59B
Average	16.54	18.12	21.37	23.54	

Description: Values with the same letter towards column (uppercase) and the values with the same letter in the direction of the line (lowercase) indicates not significantly different ($P > 0.05$)

The analysis exhibited that MDA levels in the culture conditions stress and without stress were significant different ($P < 0.05$) (Table 3) at all doses of isoflavones, except for dose at 3000 ppm. In administration of isoflavone at dose 3000 ppm, the MDA levels on stress condition was 1.74 while on without stress condition was 2.32 picomol/ml and both were no significant different ($P > 0.05$).

Table 3. The number of malondialdehyde in T-cells that cultured under oxidative stress and without oxidative stress conditions.

Culture condition	MDA levels at different doses of isoflavones (ppm)				
	0	1000	2000	3000	Average
Non-stress	2.23Aa	1.93Aa	1.84Aa	1.74Aa	1.93A
Stress	4.38Ba	3.99Ba	3.60Ba	2.32Ab	3.57B
Average	3.30	2.96	2.72	2.03	

Description: Values with the same letter towards column (uppercase) and the values with the same letter in the direction of the line (lowercase) indicates not significantly different ($P > 0.05$)

The analysis showed that SOD levels in oxidative stress condition was lower than the without oxidative stress condition and were significantly different ($P < 0.05$), except for isoflavones at dose 3000 ppm, SOD levels was not significantly different ($P > 0.05$) (Table 4). This suggests that the administration of isoflavones can significantly increase the SOD levels.

Table 4. The average number of superoxida dismutase activities in T-cells that cultured under oxidative stress and without oxidative stress conditions.

Culture condition	Superoxida dismutase activities at different doses of isoflavones (ppm)				
	0	1000	2000	3000	Average
Non-stress	23.78Aa	25.63Aa	27.48Aa	31.19Aa	27.02A
Stress	16.37Ba	17.61Ba	19.46Ba	26.25Ab	19.92B
Average	20.08	21.62	23.47	28.72	

Description: Values with the same letter towards column (uppercase) and the values with the same letter in the direction of the line (lowercase) indicates not significantly different ($P > 0.05$)

4. Discussion

Effect of isoflavone administration on percentage of CD4 T-cell is dependent on lymphocyte culture condition (condition stress and without oxidative stress). The number of CD4 lymphocyte cells on administration isoflavone at dose: 0, 1000, 2000, and 3000 ppm on culture that conditioned without oxidative stress were 19.64; 21.56; 23.91 and 25.64 respectively. These results indicate that the amount of CD4 lymphocyte cells increases in a line the increase doses of isoflavones. The Duncan test results showed that administration of isoflavones at dose 3000 ppm in without stress conditions was significantly different ($P < 0.05$) compared with the other doses. Administration of isoflavones at dose 2000 ppm was no significant different ($P > 0.05$) compared with dose 1000 ppm. However, when they compared to administration of isoflavone at dose 0 ppm, there was a significant different ($P < 0.05$).

In contrast, administration of isoflavones on oxidative stress conditions, resulting in percentage number of CD4 T cells was 11.42; 13.15; 15.58 and 18.38, respectively. When it counted the CD4 T-cells that under stress condition was lower than the without stress condition. This suggests that paraquat as initiated the oxidative stress is able to decrease the number of CD4 T-cells. Additionally, and administration of isoflavones has not been able to maintain and restore the number of CD4 T-cells even in the without stress condition.

In general, administration of isoflavones could increase amount of CD4 lymphocyte cells and this something to do with the isoflavone antioxidative capability in protecting T-cells from free radical or toxical effects of paraquat so that the amount of lymphocyte cells was not disrupted. Kartikawati (1999) showed that rats with paraquat administration caused decrease amount of CD3 and CD4 lymphocyte cells. In addition, administration of antioxidants, such as vitamin C and E was able to protect lymphocytes from the toxical effects of paraquat.

Similar to CD4, CD8 T-cell count is dependent on the condition of lymphocyte culture. In without oxidative stress conditions and without administration of isoflavones (0 ppm) the amount of CD8 T-cells was 21.15, while administration of isoflavones at dose: 1000, 2000 and 3000 ppm, percentage number of CD8 T-cells was 22.45; 25.76, and 27.42, respectively (Table 2). Under oxidative stress conditions, isoflavones prevented the decrease amount of CD8 lymphocyte cells The increase of isoflavone gives better prevention capability, which is characterized by higher number of CD8 T-cells compared with the number of CD8 T-cells on under stress conditions without isoflavones administration. The involvement of amount of CD8 T-cells is important in the role of antioxidative isoflavones which is able to neutralize and destroy free radicals. Thus, they prevent damage to T-cells so that it does not impact to the number of CD8 T-cells.

Effect of isoflavone administration on MDA T-cells levels is dependent on lymphocyte culture condition (stress and without oxidative stress). In without oxidative stress condition, MDA T-cell level was decreased when the dose of isoflavones was increased. Even though this condition was not significantly different ($P > 0.05$). This can be seen in the treatment of without stress conditions in which isoflavones could decrease the number of MDA T-cells at 1.93; 1.84 and 1.74 picomol/ml, respectively (Table 3). Under oxidative stress conditions and not given isoflavones, the MDA level was 4.38 picomol and it was higher compared with other treatments. This suggests that paraquat is highly toxic and generated free radicals that caused lipid peroxidation and produced high MDA

product. Isoflavones at dose 3000 ppm was significantly decreased ($P < 0.05$) the MDA levels compared to the doses of 1000 and 2000 ppm. This shows that the role of isoflavones as antioxidants can neutralize free radicals that prevent a peroxidation reaction. Thus, it prevents lipid peroxidation reaction so that MDA products are also decreased.

According to Rimbach *et al.*, (2003), isoflavones have high antioxidant activity. As antioxidants, isoflavones are able to decide by means of a chain reaction with of lipid radicals react and neutralize into a more stable product. Isoflavones play a role in eliminating free radicals which are normally produced in the body or free radicals that come from outside the body. The ability of isoflavones as antioxidants is complex and this can be seen from the structure owned by the isoflavone compounds. Apak *et al.* (2007) reported that, the structure of flavonoids, including isoflavones, contributes to the antioxidative activity. The mechanism of antioxidant activity of isoflavones is closely related to the structure and highly dependent on the number and location of the OH group (Heijnen *et al.*, 2002). Isoflavones have a hydroxyl group (-OH), the 4'-OH in the B ring (B-ring) and the OH group on the ring AC (AC-ring). Thus, the mechanism action of isoflavones is to terminate the propagation site of free radicals (free radical chain breaking), in which all of the hydroxyl groups can donate an electron or hydrogen resulting in a clearance or interception against free radicals in order to form compounds that are relatively more stable (non-radical).

SOD activity is dependent on lymphocyte cell culture conditions and level doses of isoflavones. On the group of cell culture in without stress condition and untreated isoflavones (0 ppm), the SOD activity was of 23.78, while in the same conditions but treated with isoflavones doses of 1000, 2000, and 3000 ppm had increase SOD activity at 25.63; 27.48, and 31.19 U/ml, respectively. On the other hand, for the group of cell culture in stress conditions and without isoflavone treatment (0 ppm) gave SOD value at 16.37. While, giving isoflavone at dose 1000; 2000, and 3000 ppm affected SOD activity at 17.61, 19.46, and 26.25 U/ml respectively (Table 4)

Treatment of various concentrations of isoflavones in cell cultures without the stress condition affected increased SOD activity in a line with of isoflavones increase dose. Similarly, lymphocyte cell cultures on stress conditions and treated with isoflavones, SOD activity can be maintained and even increased a line with the increase dose of isoflavones treatment. However, SOD activity was much greater in the cell culture on without stress conditions compared to cell culture on stress condition although both were treated with isoflavones. The increased of SOD activity was associated with the ability of isoflavones as antioxidants (Rimbach, 2001). Superoxide dismutase (SOD) is an intracellular enzyme that is present in the cytosol. SOD plays an important role in catalyzing dismutation reaction of radical superoxide ions to produce hydrogen peroxide and molecular oxygen (Halliwell, 2006).

Isoflavones work synergistically with SOD, in helping to neutralize free radicals that generated by paraquat so that SOD activity did not decrease. Isoflavones can stimulate intracellular antioxidant enzymes by regulating gene expression as it reported by Badeau (2008). Genestein isoflavones can increase expression of glutathione peroxidase (GPx) and the mechanism of gene transcription through the activation of NRF (nuclear related factor). The similar research was reported by Borrás *et al* (2006), isoflavones genestein is also able to increase MnSOD expression through interaction with estrogen receptors in cells and subsequent activation of ERK1/2 (extracellular-signal-regulated kinase).

The SOD, glutathione peroxidase (GPx), and catalase enzymes are antioxidant cellular enzymes that function as the major defense in eliminating free radicals (Valko *et al.*, 2007). This reaction is known as free radical scavenger reaction (Free Radical Scavenger)

5. Conclusion

Treatment of isoflavones at dose 3000 ppm on lymphocyte cell culture on oxidative stress conditions may prevent decrease number of CD4, CD8 T-cells, malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activities.

Acknowledgement

The authors wish to thank to the Higher Education Directorate (DP2M Dikti) Ministry of Education and Culture, Indonesia Government which has funded this research through University of Udayana (letter of research agreement no: 175.66/UN14.2 / PNL.01.03.00/2013, Dated May 16, 2013).

References

- Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S.E., Bektaşoğlu, B., Berker, K.I., and Özyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the cupric assay. *Molecules* 12 :1496-1547.
- Astuti, M., Bachelor, Meliala, A., Dalais, F.S., and Wahlqvist, M.L. (2000). Tempe, a nutritious and healthy food from Indonesia. *Asia Pacific J Clin Nutr.* 9(4): 322–325
- Badeau, M. (2008). Genistein And 17 β -estradiol Fatty Acid Esters And The Structure related Antioxidant Activity Of Estrogens On Lipoproteins. [Dissertation]. Helsinki. The Faculty of Medicine, University of Helsinki. 57 Hlm.
- Borra´s, C., Gambini, J., Go´mez-Cabrera, M.C., Sastre, J., Pallardo, F.V., Mann, G.E., and Vin˜a, J. (2006). Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NF κ B. *FASEB J* Vol. 20:1476-1481.
- Capeyron, M.F.M., Julie, C., Eric, B., Jean, P., Jean, M.R., Piere, B., Claude, L.L., and Benard, D. (2002). A diet cholesterol and deficient in vitamin E induces lipid peroxidation but does not enhance antioxidant enzyme expression in rat liver. *J Nutr Biochem* 13:296-301.
- Fisher, G. (2010). Oxidative Stress and Antioxidant Defenses in Lymphocytes Following High Intensity Interval Training. A dissertation submitted to the Graduate Faculty of Auburn University. Auburn, Alabama.
- Fisher, G., Schwartz, D.D., Quindry, J., Barberio, M.D, Foster, E.B., Jones, K.W., and Pascoe, D.D. (2011). Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise. *J Appl Physiol* 110: 730–737.
- Gautam, N., Das, S., Mahapatra, S.K., Chakraborty, S.P., Kundu, P.K, and Roy, S. (2010). Age associated oxidative damage in lymphocytes. *Oxidative Medicine and Cellular Longevity* 3:4, 275-282.
- Halliwell, B. (2006). Reactive spesies and antioxidants: Redox biology is a fudamental theme of aerobic life. *Plant Physioly* 141:312-322.
- Heijnen, C.G.M., Haenen, G.R.M.M., Oostveen, R.M., Stalpers, E.M., and Bast, A. (2002). Protection of flavonoid against lipid peroxidation: the structure activity relationship revisited. *Free radic Res* 36:575-581.
- Jenkins R.R. (2000). Exercise and oxidative stress methodology: a critique. *Amer J Clinical Nutr* 72: 670S-674S.
- Jezek P, and Hlavata L. (2005). Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Int J Biochem Cell Bio* 37: 2478-2503.
- Kartikawati, D. (1999). Studi Efek Protektif Vitamin C dan E Terhadap Respon Imun dan Enzim Antioksidan pada Mencit yang di Papar Paraquat Thesis. Program Pascasarjana, IPB, Bogor.
- Kelly, K. (2007). Understanding the Immune System: How It Works. Institute of Allergy and Infectious Diseases U.S. Department Of Health And Human Services National Institutes Of Health. National. NIH Publication No. 07-5423. 113 pages
- Krejsa, C.M., Schieven, G.L. (2000). Detection of Oxidative Stress in Lymphocytes Using Dichlorodihydrofluorescein Diacetate. *Methods in Molecular Biology.* Vol. 99:35-47
- Lee, J., Renita, M., Fiorito, R.J., St.Martin, S.K., Schwartz, S.J., and Vodovotz, Y. (2004). Isoflavone characterization and antioxidant activity of ohio soybeans. *J Agric Food Chem* 52:2647-1651.
- Mantik, A.N., Hartaningsih, N., Darma, D.M.N., Tenaya, W.M., Budiantono, dan Ekaana, W. (2005). Replikasi virus penyakit jembrana pada kultur limfosit darah tepi asal sapi bali. *JVet.* Vol 6 (4):135-142
- Nebot, C., Moutet, M., Huet, P., Xu, J.Z., Yadan, J.C., and Chaudiere, J. (1993). Spectrophotometric assay of superoxide dismutase activity based on the activated autooxidation of a tetracyclic catechol. *Analytical Biochem.* 214:442-451.
- Ogamba, E.N., Inyang, I.R., and Azuma, I.K. (2011). Effect of Paraquat Dichloride on Some Metabolic and Enzyme Parameters of *Clarias gariepinus*. *Curr. Res. J. Biol. Sci.*, 3(3): 186-190.
- Raina, S., Kumar, V., Kaushal, S.S., and Gupta, D. (2008). Case report: Two Cases of Paraquat Poisoning from Himachal Pradesh. *JACM* 9(2): 130-132.

- Rimbach, G., De Pascual-Teresa, S., Ewins, B.A., Matsugo, S., Uchida, Y., Minihane, A.M., Turner, R., VafeiAdou, K., Weinberg, P.D. (2003). Antioxidant and free radical scavenging activity of isoflavone metabolites. *Xenobiotica* 33:913-925.
- Shi, Y., Devadas, S., Greenelch, K.M., Yin, D., Mufson, R.A., and Zhoua, J. (2003). Stressed to death: Implication of lymphocyte apoptosis for psychoneuroimmunology. *Brain, Behavior, and Immunity*. 17:S18–S26.
- Suarsana, N., Bintang, M., Priosoeryanto, B.P., dan Wresdiyati, T. (2010). The Effects of Tempe Isoflavone On Intracellular Antioxidant Enzymes And Malondialdehyde Level In Diabetic Rat Pancreatic Islets. Abstract International Conference, Exhibition & Short Course On Nutraceuticals And Functional Foods. Sanur, Bali 11-15 October, 2010
- Todd, I. (2001). Cells of the Immune System. *Encyclopedia Of Life Sciences*. Nature Publishing Group/www.els.net. 7 page.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., and Telser, J. (2007). Review: Free radicals and antioxidants in normal physiological functions and human disease. *Inter J Biochem & Cell Biol* 39:44-84.
- Zakaria, F., Khatib, A., Hartoyo, A. (1997). Bioactive Compounds of Food and Agriculture Products Having Immunomodulator Activities. Di dalam Hariyadi RD, Zakaria F, Editor. Seminar Proceeding Biomoleculer Reaction and Industrial Applications of Immunology in Food and Agriculture. Nopember 7, 1997 Bogor. Pusat Studi Pangan dan Gizi IPB dan Kedubes Perancis-Jakarta.
- Zakaria, F., Meilasanti, M.A., Sandjaya, S., Madaniyah-Parmudya, S., dan Richards, A.L. (1997a). Aktivitas proliferasi limfosit darah tepi konsumen makanan jajanan di Bogor, Jawa Barat. *Bul. Teknol dan Industri Pangan*. Vol. VIII (2):57-65.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
<http://www.iiste.org>

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: <http://www.iiste.org/journals/> All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: <http://www.iiste.org/book/>

Recent conferences: <http://www.iiste.org/conference/>

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar

